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(54) Title: XYLANASE, OLIGONUCLEOTIDIC SEQUENCE ENCODING IT AND ITS USES

(57) Abstract

Xylanase stable at 60 °C and having a sequence comprising 182 aminoacids, and oligonucleotidic sequence encoding it. This oligonucleotidic sequence, comprised in an appropriate vector, allows the production of xylanase.

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Xylanase, oligonucleotidic sequence encoding it and its uses

The present invention relates to a xylanase and to a nucleotidic sequence encoding it.

It also relates to the use of this enzyme in the bleaching of paper pulp and the preparation of xylose or of xylo-oligosaccharides from plant raw materials, in particular.

Varied uses have been proposed for xylanases in the biotechnology field, especially in the foodstuffs field (Biely, Trends Biotechnol 3 (11): 286-290, 1985), in the paper industry (Mora et al., J. Wood Chem. Technol, 6: 147-165, 1986) or in the production of chemical compounds from hemicellulose (Reilly P.J., 1981, Xylanases: structure and function in trends in the biology of fermentations for fuels and chemicals. A.J. Hollaender (Ed), Plenum, New York).

The technical feasibility of such applications has been assessed chiefly using enzymes produced by mesophilic fungi. However, such applications could be facilitated by the use of fungi possessing better temperature stability.

Various bacteria and enzymes are known for the production of xylanases (see, in particular, Wong et al., Microbiological Reviews, 52, No 3 305 317, 1988). Hitherto, the highest yields of enzymes have been obtained from fungi (Yu et al. Enzymo Microb. Technol. 9: 16-24, 1987). However, hyperproductive strains of *Bacillus* have already been described (Okazaki et al. Appl. Microbiol. Biotechnology, 19: 335-340, 1984; Okazaki et al., Agric. Biol. Chem. 49, 2033-2039, 1985). Such thermophilic species of *Bacillus* which degrade xylan can be good

candidates for the industrial production of xylanases on account of their high growth rate and of their genetics being well known.

The xylanases isolated by Okazaki et al. emanate from two *Bacillus* strains referred to as W1 and W2 by the authors. In each of these strains, two components of the xylanase activity, referred to as I and II, have been demonstrated. The components I degrade xylan to xylobiose and to oligomers having a higher degree of polymerization, while the components II produce xylose in addition to the above compounds.

The components I (referred to as W1.I and W2.I) have respective molecular weights of 21.5 kDa and 22.5 kDa, as well as isoelectric points of 8.5 and 8.3. The components II (W1.II and W2.II) have, for their part, respective molecular weights of 49.5 kDa and 5O kDa.

The two components I and II are inhibited by Hg^{++} ions and, to a lesser extent, by Cu^{++}.

Many other xylanases have been isolated from various species of *Bacillus, Clostridium, Aspergillus, Streptomyces* or *Trichoderma*, inter alia (Wong et al., 1988, cited above).

Thus, the résumé of Japanese Patent JP 130 96 84 (RIKAGAKU KENKYSHO) relates to a type WII xylanase having a molecular weight of 5O kD or 42 kD. No isoelectric point is mentioned for this xylanase.

A paper by RAJARAM et al., (Applied Microbiology and Biotechnology, Vol. 34, n°1, October 1990, pages 141-144) relates to a *Bacillus* strain isolated in the natural environment and which produces a

xylanase having optimal activity at between 6O°C and 7O°C and at a pH of between 6 and 7. This enzyme is characterized neither by its molecular weight nor by its isoelectric point. This strain produces, in addition, other enzymes such as cellulases.

Another résumé of a Japanese patent in the name of RIKAGAKU KENKYUSHO (JP-85 118 644) describes a xylanase having optimal activity at a pH of between 6 and 7. This enzyme is considered to have a molecular weight, determined by ultrafiltration, of between 5O and 100 kD. No isoelectric point is mentioned in this résumé.

A paper by GRÜNINGER et al. (Enzyme Microbiology and Technology, Vol. 8, May 1986, pages 309-314) relates to a *Bacillus stearothermoplilus* strain isolated from mud and which produces a heat-stable xylanase. The enzyme is characterised as having optimal activity at 78°C and at a pH value of 7.5. This enzyme is characterized neither by its molecular weight nor by its isoelectric pH.

The industrial production of xylanases is impeded by the simultaneous presence of contaminant activities such as cellulases, leading to additional purification costs.

As far as the Applicant is aware, the best productivity with respect to endoxylanase obtained with a microorganism not producing cellulase has been obtained from a Streptomyces lividans mutant devoid of cellulase activity after introduction of a plasmid carrying genes coding for xylanases A and B. Productivities of the order of 6OOO to 10,000 IU.l/h were observed in the culture media. It should nevertheless be noted that, in this case, problems linked to the failure of xylanase A to

hydrolyse insoluble xylan, and of thermal stability in the case of xylanase B, were encountered (Kluepfel et al. Biochem. J. 267, 47-50, 1990).

None of these enzymes hence possessed, as far as the Applicant is aware, features making an industrial application possible, that is to say good thermal stability, a large capacity for degradation of substrates, a means of production by hyperproductive strains and possibilities to modify the aminoacids sequence.

Another xylanase has been isolated from a Bacillus strain deposited at the CNCM Culture Collection under the number I-1017. It has been described in EP 0.573.536 application filed under the name of the present applicant. This xylanase displays a temperature stability. However its sequence has not been determined and it can only be produced by growing the said Bacillus strain.

Thus it was not possible to modify its protein sequence in the aim of improving its properties.

The applicant has thus cloned the gene encoding this xylanase and has sequenced it.

The subject of the present invention is, thus a thermophilic xylanase having a sequence sharing an homology of at least 80%, preferentially 90%, and more preferentially 95%, with the following one (SEQ ID N°2):

Asn Thr Tyr Trp Gln Tyr Trp Thr Asp Gly Ile Gly Tyr Val Asn Ala Thr
Asn Gly Gln Gly Gly Asn Tyr Ser Val Ser Trp Ser Asn Ser Gly Asn
Phe Val Ile Gly Lys Gly Trp Gln Tyr Gly Ala His Asn Arg Val Val Asn
Tyr Asn Ala Gly Ala Trp Gln Pro Asn Gly Asn Ala Tyr Leu Thr Leu
Tyr Gly Trp Thr Arg Asn Pro Leu Ile Glu Tyr Tyr Val Val Asp Ser Trp

Gly Ser Tyr Arg Pro Thr Gly Asp Tyr Arg Gly Ser Val Tyr Ser Asp Gly Ala Trp Tyr Asp Leu Tyr His Ser Trp Arg Tyr Asn Ala Pro Ser Ile Asp Gly Thr Gln Thr Phe Gln Gln Tyr Trp Ser Val Arg Gln Gln Lys Arg Pro Thr Gly Ser Asn Val Ser Ile Thr Phe Glu Asn His Val Asn Ala Trp Gly Ala Ala Gly Met Pro Met Gly Ser Ser Trp Ser Tyr Gln Val Leu Ala Thr Glu Gly Tyr Tyr Ser Ser Gly Tyr Ser Asn Val Thr Val Trp

The degree of homology can be determined using pairwise alignment methods such as the GAP and the BESTFIT programs of the Genetics Computer Group, Inc. Package (GCG) Fast database searching programs such asd FASTA and BLAST (included in the GCG package) can be used for the comparison of a sequence to all available sequences of a database.

For the definition of the term "homology" one can refer to Altschul et al. (1990, J. Mol. Biol. 215, 403-410) and Doolittle R.F. (Ed) (Molecular evolution: computer analysis of protein and nucleic acid sequences. Methods in Enzymology 183, Academic Press, London, 1990).

Xylanases falling under this definition are in particular the ones in which one or a few aminoacids have been changed, compared to the sequence SEQ ID N°2.

Such changes in the aminoacids are preferentially the ones which consist in the substitution of one aminoacid by another one which has substantially the same properties such as defined by Lehninger (page 70, 2nd french edition, 1979, Flammarion ed.) or in its more recent re-edition. It is reminded that the twenty basic aminoacids are classified in four groups depending on their properties:

- the ones having a hydrophobic or non-polar lateral chain,

- the ones having a polar lateral chain not charged,
- the ones having a negatively charged lateral chain, and
- the ones having a positively charged lateral chain.

Such a xylanase can possess a molecular mass of approximately 22 kDa, determined by SDS-PAGE, or 20.7 kDa determined by mass spectroscopy and an isoelectric point of approximately 7.7.

This enzyme advantageously displays great stability at 60°C for at least 24 hours, and a pH of optimal activity within the range extending from 4.8 to 7, and preferably approximately 6.

It should be noted that the pH_i of this enzyme is fairly high but nevertheless lower than the pH_i of the xylanases of similar molecular mass produced by some bacilli, in particular those described by Okazaki et al. (1985, publication cited above).

pH 6 corresponds to an optimal pH, but the activity remains greater than 80% in the range between 4.8 and 7.

Another subject of the present invention is a nucleotidic sequence coding for the said xylanase. This sequence can be DNA or RNA sequence and in particular c DNA, plasmidic DNA, genomic DNA or m RNA.

Preferentially such a nucleotidic sequence is the following one (SEQ ID N°1):

aacacgtactggcagtattggacggatggcatcgggtatgtgaacgcgacgaacggaca
aggcggcaactacagcgtaagctggagcaacagcggcaacttcgtcatcggcaagggct
ggcaatacggtgcgcacaaccgggttgtcaactacaacgccggcgcatggcagccgaa
cggcaacgcgtatctgacgctgtacggctggacgcgcaacccgctcatcgaatactacgt

**cgtcgacagctggggcagctaccgcccgaccggcgactaccggggcagcgtgtacagc
gacggcgcatggtatgacctctatcacagctggcgctacaacgcaccgtccatcgacggc
acgcagacgttccaacaatactggagcgttcgtcagcagaaacgcccgacgggcagcaa
cgtctccatcacgttcgagaaccacgtgaacgcatggggcgctgccggcatgccgatgg
gcagcagctggtcttaccaggtgctcgcaaccgaaggctattacagcagcggatactcca
acgtcacggtttggtaa**

The xylanase according to the present invention can thus also be produced by a microorganism strain, appropriately chosen, transformed by a vector coding for the said xylanase. The said microorganism is grown in an appropriate medium and thereafter the xylanase is isolated as described below.

Such a microorganism is chosen in order to be able to produce and to excrete it.

It can be a bacteria such as *Escherichia coli* or *Bacillus sp.*

The vector coding for the xylanase is chosen in order to be expressed in the said microorganism. It can be a plasmid, such as pBluescript or preferentially pET..

Systems of expression suitable for the production of the xylanase according to the present invention are in particular listed in D.V. Goeddel ((Ed). Gene expression technology. Methods in Enzymology, 185, Academic Press, London, 1990).

The pET E.coli expression system is one of the most widely used bacterial expression system (Studier et al., 1990, Meth. Enzymol., 185, 6O-89).

The expression of recombinant xylanase can be achieved in particular as following. The DNA fragment encoding the mature

xylanaxe, i.e. the sequence SEQ ID N°1, is engineered by PCR so as to generate NdeI and BamhI terminal restriction sites suitable for expression in the T7-based vector pET3a. The PCR fragment is cloned blunt-ended into pBluescript (Stratagene Cloning Systems) before cloning as a NdeI/BamHI fragment into pET3a.

The recombinant enzyme is expressed from pET3a in the E. coli strain BL21 (DE3) carrying pLysS. Cultures are grown in L-broth containing ampicillin (100 µg/ml) and chloramphenicol (25 µg/ml) until an A_{600} of 3 was reached, before induction with 0.1 mM isopropyl ß-D-thiogalactoside (IPTG) for 3 hours. Large-scale cultures for protein purification are centrifuged and the cells are lysed in a buffer containing 5O mM Tris-HCl, pH 8.O, 1 mM EDTA by passage through a French press (10 MPa). The same process described in EP 0.573.536 for purifying the xylanase from the culture supernatant of the *Bacillus* can be used. One can expect about 1 mg of recombinant protein per ml of cell culture.

An advantage of this way of production of the xylanase is that the nucleoditic sequence can be mutated before to be introduced in the microorganism. It is therefore easy to obtain various mutations corresponding to xylanases having various sequences.

This was not possible with the thermostable xylanases already described in the prior art, such as the one described by GRÜNINGER et al. (previously cited), since their sequences were not known.

For carrying out the present invention, in particular this way of production, the man skilled in the art can refer to the following manual which describes the usual techniques of molecular biology: Maniatis et

al. 1982- Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Ed. New York, or one of its more recent editions.

The xylanase as described above can be obtained through a process, comprising the following steps:

- concentration of the microorganism culture supernatant,

- passage through an ion exchange column such as a column of Q Sepharose Fast Flow (Pharmacia),

- passage through a hydrophobic interaction column such as a column of Phenyl-Sepharose (Pharmacia).

Concentration of the supernatant can , in particular, be performed by ultrafiltration through a polysulphone membrane having an exclusion threshold above 10 kDa.

This process enables a substantially pure xylanase preparation to be obtained.

The xylanase described above can be produced through a process comprising the steps:

- of growth of the bacteria in a medium containing a growth substrate such as glucose, and

- of production of xylanase induced by feeding the culture continuously with suitable amounts of xylo-oligosaccharides.

The subject of the present invention is also the use of the xylanase described above in the bleaching of paper pulp.

An advantage of this xylanase lies in the fact that the degree of hydration of the paper pulp is of little importance. It is not obligatory to dilute the pulp greatly in order to obtain good enzymatic attack. The use of this xylanase as an auxiliary in the bleaching of paper pulp is all the

more advantageous for the fact that the preparations are devoid of cellulase contaminants.

This xylanase may also be used for the preparation of xylose or of xylo-oligosaccharides from raw materials of plant origin, which are inexpensive and renewable raw materials (for example maize cobs).

Other uses of xylanases have been mentioned in the literature. The review by Zeikus et al. (Thermostable saccharidases New Sources uses and Biodesigns in "Enzymes in biomass conversion", Leatham and Himmel, ACS Washington D.C. , 1991) lists the main uses of xylanases. They are mainly used in food manufacture, where their properties enable bread-making, the clarification of fruit juices and wines and the nutritional qualities of cereal fibres to be improved, and in the production of thickeners for foodstuffs.

The second sphere of application relates to the paper pulp and fibre industries, where they are used for the bleaching of pulps, the manufacture of wood pulp and the purification of fibres for rayon manufacture.

Uses are also noted in poultry feeding, in which uses xylanases are employed in order to decrease the viscosity of the feeds (Van Paridon et al. Xylans and Xylanases, International Symposium, Wageningen, 8-11 December 1991; Bedford and Classen H.L Xylans and Xylanases, International Symposium, Wageningen, 8-11 December 1991).

The use of xylanase in enhancing the value of by-products of the paper pulp industry is more specifically mentioned in the paper by Biely (Trends in Biotechnology, Vol.3, No.11, 1985).

Mention may also be made of the two European Patents EP 228,732 and EP 227,159, which relate to the use of Xylanases for improving the filterability of glucose syrup and of beer, respectively.

The possibility of using the xylanases for the production of chemical compounds from hemicellulose (Reilly, cited above) will also be noted.

These various publications show that the xylanases which are the subject of the present invention may be used in a large number of applications.

The present invention is illustrated, without, however, being limited, by the application examples which follow.

Fig. 1 illustrates the homology degree between the xylanase according to the present invention (XYL2O) and other xylanases.

Fig.2 represents HCA plots of four xylanases, including the one of the present invention.

On fig.3 is indicated the prediction of secondary structural elements for the xylanase of the present invention.

EXAMPLE 1:

Cloning and sequencing of the gene encoding for the xylanase.

1. Materials and methods.

- **Strains, vectors and culture conditions.**

The strain I-1017 was grown at 55°C in the liquid medium described in examples 1 and 2 of patent application EP-0.573.536. The SURE, XL1-Blue and XLOLR Escherichia coli strains, the vectors ZAP Express and pBluescript and the filamentous helper phage ExAssist™

were all purchased from Stratagene Cloning Systems E. coli cells were grown in LB medium at 37°C. The medium was solidified by addition of 1.5% (w/v) of Bacto-agar.

- **<u>Preparation of DNA</u>**

Bacterial genomic DNA was extracted from I-1017 according to the method of Yang et al. (Appl. Environ. Microbiol., 1988, 54, 1023-1029).

- **<u>Obtention of a partial genomic clone coding for the XYL2O</u>**.

PCR was used to amplify a region of the chromosomal DNA coding for the XYL2O. The nucleotide sequence of the forward primer (P1) (SEQ ID N°3) was AAYACNTAYTGGCARTAYTGGACNGAYGG (derived from the sequence NTYWQYWTDG in the N-terminus end of the XYL2O); that of the reverse primer (P2) (SEQ ID N°4) was YTGWCKNACRCTCCARTAYTG (corresponding to the sequence QYWSVRQ, a conserved region near the C-terminus of other xylanases from different *Bacillus* species). PCR was performed with chromosomal DNA as a template and the primers P1 and P2 on a thermocycler (Perkin-Elmer. France) with the following temperature profile: 1 min 94°C -1 min 5O°C - 2 min 72°C for 35 cycles. The PCR product was purified on a 1% agarose gel and was ligated into EcoRV-digested pBluescript. The chimaeric plasmid (pBX2O) was used to transform SURE cells. Recombinant cells were selected on L-agar plates containing ampicillin (4O µg/ml), isopropyl-ß-D-thiogalactoside (O.2 mM) and 5-bromo-4-chloro-3-indolyl-ß-D-galactoside (4O µg/ml).

- **Construction of a B. sp I-1017 genomic library in ZAP Express.**

Chromosomal DNA was partially digested with Sau3AI and the resulting DNA fragments in the size range 1.5-8 kb were purified and ligated into BamHI-digested ZAP Express. The library was constructed using XL1-Blue cells as indicated by the manufacturer.

- **Screening of the genomic library**.

pBX2O was digested with BamHI and HindIII and the DNA insert was purified and labeled with digoxigenin (Boehringer Mannheim) following the instructions of the manufacturer. The labeled DNA was used to screen the genomic library. After the third screening, positive lambda plaques were isolated and the recombinant plasmid pBK-CMV inserted in the vector ZAP Express was excised using the filamentous phage ExAssist and then recovered by infecting the XLOLR cells in the presence of kanamycin (10 µg/ml).

- **DNA sequence analysis**.

Plasmid preparations for sequence determination were performed using Qiagen tip 100 (Diagen, Coger, France). Double-stranded DNA sequencing was done by the dideoxy chain termination method of Sanger et al (Proc. Nat. Acad. Sci. USA, 1977, 74, 5463-5467), using the Sequenase™ 2.O DNA sequencing kit from United States Biochemical . Both universal and specific primers were used to sequence the sense and antisense strands of inserts in the plasmids.

- **Protein sequence analysis and Hydrophobic Cluster Analysis**.

The sequence Analysis Software Package by Genetics Computer, Inc. (The GCG Package) was used throughout this work. In particular, multiple alignments were performed using the Pileup program and pairwise comparisons were done using the Bestfit program.

Hydrophobic Cluster Analysis (HCA) is a method to compare amino acid sequence (Gaboriaud et al. FEBS Lett., 1987, 224, 149-155) which is derived from the theory of Lim (J. Mol. Biol, 1974, 88, 857-872). The method involves the drawing of the sequence of a theoretical Â-helix where the hydrophobic residues form clusters. The shape, size and the relative position of the clusters can be compared and the sequence similarity, when it exists, may be readily revealed. Conversion of the amino acid sequences into the 2D-helical plot required by the method was made using the HCA-Plot software.

2) **Results**.

In a first attempt to determine the xylanase sequence, the xylanatic activity has been tested in the genomic library. However this approach has failed to conduct to the isolation of the xylanase according to the present invention.

In a second and successful attempt, the sequence of this xylanase has finally been determined.

It is reminded that the amino acid sequence of the N-terminal region of the xylanase has been determined (example 4 of EP 0.573.536). It exhibits 67% of identity with the N-terminus of the xylanases produced by *Bacillus subtilis* and *Bacillus circulans*.

Besides, these enzymes which belong to the G family (according to the classification of Gilkes et al. (Microbiol. Rev., 1991, 55,

303-315)) share some conserved regions along their polypeptide chains. Among others, one region consisting of 7 amino acids occurs near the C-terminus.

A part of the gene coding for the xylanase has been amplified by PCR using two degenerate primers, P1 and P2, corresponding to the N-terminus end of the xylanase and to a conserved region near the C-terminus, respectively. A 45O bp DNA fragment was obtained and cloned into the vector pBluescript. The sequence of the resultant plasmid pBX2O can be attributed without any doubt to the xylanase. To get the complete gene of xylanase, a genomic library of B. sp I-1017 was prepared in E. coli XL1-blue using the phage vector ZAP Express. This library was screened with the insert of the plasmid pBX2O. One positive plaque, designated pBX52Â2, was shown to contain the complete gene of the xylanase.

The nucleotidic sequence of this clone is indicated in the sequence list hereunder as SEQ ID N° 1.

The complete protein sequence of the xylanase is shown as SEQ ID N°2 is the sequence list hereunder.

The results of a FASTA search in the protein data bases PIR and Swiss-Prot yielded 36 xylanase sequences. As shown in the table the xylanase shares sequence homology with other xylanases of the G family. The best scores (73% of identity) are observed as expected with the xylanases from B. subtilis and B. circulans. This shows unambiguously that the xylanase according to the present invention is a new protein which possesses a unique amino acid sequence.

For comparative purposes, only representative xylanases from different organisms (the ones in bold types on the table) are listed in the multiple sequence alignment shown in figure 1. The analysis of a primary sequence alignment of 14 xylanases of the G family indicates the residues which are conserved throughout the family. As reported previously by Wakarchuk et al., (Protein Sci., 1988, 3, 467-475), 2 glutamic acid residues are absolutely conserved in this family of xylanases. The present multiple alignment suggests that Glu76 and Glu169 are the catalytic residues of the xylanase.

Alignment was then reconsidered by the HCA method (Gaboriaud et al., 1987, previously cited), which allows for a rapid identification of the clusters and an easy alignment (figure 2). The identification of the clusters is straightforward even if there are some variations in cluster shapes. The alignment was checked on crystalline structures for three xylanases indicated in figure 1, and the extensions of the ß-strands are indicated on the HCA plots. Vertical lines have been inserted to delimit the extension of ß-strands. The most conserved hydrophobic clusters have been shadowed for better visualization.

For the HCA plot of xylanase according to the present invention (XYL2O), the extension of the ß-strands was deduced from the other plots. It appears clearly that the secondary structure of XYL2O consists essentially of ß-strands and only one Â-helix. These elements are so organized to display the characteristic folding of a greek key. We can also inferred that the following aromatic residues: Tyr 67 and Tyr 78 on one ß-strand and Tyr 163 on another ß-strand are likely to be involved in

the orientation and binding of xylan polysaccharides, prior their hydrolysis.

The figure 3 summarizes the prediction of the occurrence of secondary structural elements which can be proposed for the xylanase according to the present invention on the basis of its primary structure and a thorough protein sequence analysis. These structural predictions can be translated into a putative three-dimensional model to be used in Molecular Isomorphism Replacement in view of solving the crystalline structure of this xylanase.

Table Summary of xylanases derived from a FASTA search in the Protein Data Bases Swiss Prot (release 31.0) and PIR (release 44.0).

AC (1)	date	origin		cryst	%	nb a.a	Mw
P18429	01.11.90	*Bacillus subtilis*	XYNA		73	213	23,345
S39157	18.02.94						
P09850	01.03.89	*Bacillus circulans*		*	73	213	23,345
S01734	07.06.90						
P26220	01.02.95	*Streptomyces lividans*	XYLC		60	240	25,673
JS0591	14.07.94						
S47512	13.01.95	*Streptomyces. sp EC3*			58	240	
JS0590	10.03.94	*Streptomyces lividans*	XYLB		58	333	
P26515	01.08.92		XYNB		58	333	35,426
S43919	25.10.94	*Humicola insolens*			55	227	
A44594	14.06.94	*Trichoderma viride*	XYL II A		54	190	
A44595	27.06.94		XYL II B		54	190	
P36217	01.06.94	*Trichoderma reesei*	XYN2		54	222	24,172
S39154	06.03.94		XYL I				
S39883	27.05.94		XYL II	*			
P35809	01.06.94	*Schizophyllum commune*			54	197	20,978
A44597	27.06.94						
S38973	18.02.94						
Q06562	01.02.95	*Cochliobolus carbonum*			53	221	23,728
A44593	27.06.94	*Trichoderma harzianum*			53	190	
P00694	21.07.94	*Bacillus pumilus*	XYNA	*	50	228	25,521
WWBSXP	20.09.84						
JQ1935	20.08.94	*Clostridium stercorarium*	XYL A		48	511	
P33558	01.02.95				48	511	56,519
P17137	01.08.94	*Clostridium acetobutylicum*			46	261	29,032
S12745	30.09.93						
P33557	01.02.94	*Aspergillus awamori*	XYNC		40	211	22,560
S43015	20.05.94				40	211	
JC1198	05.03.93	*Aspergillus niger*	XYNC		40	211	
P36218	01.06.94	*Trichoderma reesei*	XYN1		39	229	24,583
S39155	06.03.94		XYL2				
S48865	13.01.95	*Neocallimastix frontalis*			37	266	
S49528	20.02.95				35	607	
S24754	30.09.93	*Neocallimastix patricium*	XYL A		35	607	
P29127	01.12.92		XYNA		34	607	66,175
P35811	01.02.95	*Fibrobacter succinogenes*			34	608	66,415

(1) : AC : Accession Number
(2) : cryst : crystallographic data available
(3) : % : percentage of identity
Only the sequences in bold have been used in the alignment study, on figure 2

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE(INRA)
(B) STREET: 147, rue de l'Universite
(C) CITY: PARIS
(E) COUNTRY: FRANCE
(F) POSTAL CODE (ZIP): 75341
(G) TELEPHONE: 42 75 90 00
(H) TELEFAX: 42 75 94 28

(ii) TITLE OF INVENTION: XYLANASE, OLIGONUCLEOTIDIC SEQUENCE ENCODING IT AND ITS USES

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: US 08/543.956

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 549 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Bacillus sp
(B) STRAIN: I-1017

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION:1..547

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
AAC ACG TAC TGG CAG TAT TGG ACG GAT GGC ATC GGG TAT GTG AAC GCG      48
Asn Thr Tyr Trp Gln Tyr Trp Thr Asp Gly Ile Gly Tyr Val Asn Ala
  1               5                  10                  15
```

ACG AAC GGA CAA GGC GGC AAC TAC AGC GTA AGC TGG AGC AAC AGC GGC 96
Thr Asn Gly Gln Gly Gly Asn Tyr Ser Val Ser Trp Ser Asn Ser Gly
20 25 30

AAC TTC GTC ATC GGC AAG GGC TGG CAA TAC GGT GCG CAC AAC CGG GTT 144
Asn Phe Val Ile Gly Lys Gly Trp Gln Tyr Gly Ala His Asn Arg Val
35 40 45

GTC AAC TAC AAC GCC GGC GCA TGG CAG CCG AAC GGC AAC GCG TAT CTG 192
Val Asn Tyr Asn Ala Gly Ala Trp Gln Pro Asn Gly Asn Ala Tyr Leu
50 55 60

ACG CTG TAC GGC TGG ACG CGC AAC CCG CTC ATC GAA TAC TAC GTC GTC 240
Thr Leu Tyr Gly Trp Thr Arg Asn Pro Leu Ile Glu Tyr Tyr Val Val
65 70 75 80

GAC AGC TGG GGC AGC TAC CGC CCG ACC GGC GAC TAC CGG GGC AGC GTG 288
Asp Ser Trp Gly Ser Tyr Arg Pro Thr Gly Asp Tyr Arg Gly Ser Val
85 90 95

TAC AGC GAC GGC GCA TGG TAT GAC CTC TAT CAC AGC TGG CGC TAC AAC 336
Tyr Ser Asp Gly Ala Trp Tyr Asp Leu Tyr His Ser Trp Arg Tyr Asn
100 105 110

GCA CCG TCC ATC GAC GGC ACG CAG ACG TTC CAA CAA TAC TGG AGC GTT 384
Ala Pro Ser Ile Asp Gly Thr Gln Thr Phe Gln Gln Tyr Trp Ser Val
115 120 125

CGT CAG CAG AAA CGC CCG ACG GGC AGC AAC GTC TCC ATC ACG TTC GAG 432
Arg Gln Gln Lys Arg Pro Thr Gly Ser Asn Val Ser Ile Thr Phe Glu
130 135 140

AAC CAC GTG AAC GCA TGG GGC GCT GCC GGC ATG CCG ATG GGC AGC AGC 480
Asn His Val Asn Ala Trp Gly Ala Ala Gly Met Pro Met Gly Ser Ser
145 150 155 160

TGG TCT TAC CAG GTG CTC GCA ACC GAA GGC TAT TAC AGC AGC GGA TAC 528
Trp Ser Tyr Gln Val Leu Ala Thr Glu Gly Tyr Tyr Ser Ser Gly Tyr
165 170 175

TCC AAC GTC ACG GTT TGG T AA 549
Ser Asn Val Thr Val Trp
180

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 182 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Asn Thr Tyr Trp Gln Tyr Trp Thr Asp Gly Ile Gly Tyr Val Asn Ala
1 5 10 15

Thr Asn Gly Gln Gly Gly Asn Tyr Ser Val Ser Trp Ser Asn Ser Gly
20 25 30

Asn Phe Val Ile Gly Lys Gly Trp Gln Tyr Gly Ala His Asn Arg Val
35 40 45

Val Asn Tyr Asn Ala Gly Ala Trp Gln Pro Asn Gly Asn Ala Tyr Leu
50 55 60

Thr Leu Tyr Gly Trp Thr Arg Asn Pro Leu Ile Glu Tyr Tyr Val Val
65 70 75 80

Asp Ser Trp Gly Ser Tyr Arg Pro Thr Gly Asp Tyr Arg Gly Ser Val
85 90 95

Tyr Ser Asp Gly Ala Trp Tyr Asp Leu Tyr His Ser Trp Arg Tyr Asn
100 105 110

Ala Pro Ser Ile Asp Gly Thr Gln Thr Phe Gln Gln Tyr Trp Ser Val
115 120 125

Arg Gln Gln Lys Arg Pro Thr Gly Ser Asn Val Ser Ile Thr Phe Glu
130 135 140

Asn His Val Asn Ala Trp Gly Ala Ala Gly Met Pro Met Gly Ser Ser
145 150 155 160

Trp Ser Tyr Gln Val Leu Ala Thr Glu Gly Tyr Tyr Ser Ser Gly Tyr
165 170 175

Ser Asn Val Thr Val Trp
180

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic,degenerate oligonucleotide"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AAYACNTAYT GGCARTAYTG GACNGAYGG 29

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic,degenerate oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

YTGWCKNACR CTCCARTAYT G 21

CLAIMS:

1. Xylanase having a sequence, sharing an homology of at least 80%, and preferentially 90%, with the one having the following sequence SEQ ID N°2:

Asn Thr Tyr Trp Gln Tyr Trp Thr Asp Gly Ile Gly Tyr Val Asn Ala Thr Asn Gly Gln Gly Gly Asn Tyr Ser Val Ser Trp Ser Asn Ser Gly Asn Phe Val Ile Gly Lys Gly Trp Gln Tyr Gly Ala His Asn Arg Val Val Asn Tyr Asn Ala Gly Ala Trp Gln Pro Asn Gly Asn Ala Tyr Leu Thr Leu Tyr Gly Trp Thr Arg Asn Pro Leu Ile Glu Tyr Tyr Val Val Asp Ser Trp Gly Ser Tyr Arg Pro Thr Gly Asp Tyr Arg Gly Ser Val Tyr Ser Asp Gly Ala Trp Tyr Asp Leu Tyr His Ser Trp Arg Tyr Asn Ala Pro Ser Ile Asp Gly Thr Gln Thr Phe Gln Gln Tyr Trp Ser Val Arg Gln Gln Lys Arg Pro Thr Gly Ser Asn Val Ser Ile Thr Phe Glu Asn His Val Asn Ala Trp Gly Ala Ala Gly Met Pro Met Gly Ser Ser Trp Ser Tyr Gln Val Leu Ala Thr Glu Gly Tyr Tyr Ser Ser Gly Tyr Ser Asn Val Thr Val Trp

2. Xylanase according to claim 1, which is stable at approximately 60°C for 24 hours.

3. Xylanase according to one of the claims 1 and 2, which is secreted by a microorganism strain, appropriately chosen, transformed by a vector encoding the said xylanase.

4. Nucleotidic sequence coding for the xylanase according to one of the claims 1 to 3.

5. Nucleotidic sequence having the following sequence SEQ ID N°1:

aacacgtactggcagtattggacggatggcatcgggtatgtgaacgcgacgaacggaca
aggcggcaactacagcgtaagctggagcaacagcggcaacttcgtcatcggcaagggct

ggcaatacggtgcgcacaaccgggttgtcaactacaacgccggcgcatggcagccgaa cggcaacgcgtatctgacgctgtacggctggacgcgcaacccgctcatcgaatactacgt cgtcgacagctggggcagctaccgcccgaccggcgactaccggggcagcgtgtacagc gacggcgcatggtatgacctctatcacagctggcgctacaacgcaccgtccatcgacggc acgcagacgttccaacaatactggagcgttcgtcagcagaaacgcccgacgggcagcaa cgtctccatcacgttcgagaaccacgtgaacgcatggggcgctgccggcatgccgatgg gcagcagctggtcttaccaggtgctcgcaaccgaaggctattacagcagcggatactcca acgtcacggtttggtaa

6. Vector, in particular plasmid, comprising a sequence according to one of the claims 4 and 5.

7. Process for the production of a xylanase having the sequence SEQ ID N°2, or sharing an homology of at least 80% and preferentially 90%, with SEQ ID N°2 wherein:

- a microorganism strain appropriately chosen and transformed by a vector encoding the said xylanase according to claim 6 is grown in an appropriate medium, and

- the xylanase is isolated.

FIG.1 1/5

```
        1                                                  50
xyl20   .......... .......... .......... .......... ..........
Bac sub .......... .......... .......... ..MFKFKKNF LVG...LSAA
Bac cir .......... .......... .......... ..MFKFKKNF LVG...LSAA
Str liv .......... ....MQQDGT QQDRIKQSPA PLNGMSRRGF LGGAGTLALA
Str ec3 .......... ....MQQDGK RQDQNQQHPA PFSGLSRRGF LGGAGTVALA
Hum ins .......... .......... ........MV SLKSVLAAAT AVSSAIAAPF
Tri vir .......... .......... .......... .......... ..........
Tri ree .......... .......... ........MV SFTSLLAASP PSR....ASC
Sch com .......... .......... .......... .......... ..........
Coc car .......... .......... ........MV SFTSIITAAV AATGALAAP.
Bac pum MNLRKLRLLF VMCIGLTLIL TAV....... .......... ..........
Clo ste ....MKRKVK KMAAMATSII MAIMIILHSI .......... ..........
Clo ace ....MLRRKV IFTVLATLVM TSLTIVDNTA FAATNLNTTE STFSKEVLST
Asp awa .......... .......... .......... .......... ......MKVT

        51                                                100
xyl20   .......... .....NT... .......YWQ YWTDGIGYVN ATNGQGGNYS
Bac sub LMSISLFSAT ASAASTD... .......YWQ NWTDGGGIVN AVNGSGGNYS
Bac cir LMSISLFSAT ASAASTD... .......YWQ NWTDGGGIVN AVNGSGGNYS
Str liv TASGLLLPGT AHAATTITTN QTGTDGMYYS FWTDGGGSVS MTLNGGGSYS
Str ec3 TASGLLLPST AHAATTITTN QTGYDGMYYS FWTDGGGSVS MTLNGGGSYS
Hum ins DFVPRDNSTA LQARQVTPNA EGWHNGYFYS WWSDGGGQVQ YTNLEGSRYQ
Tri vir .......... ....QTIGPG TGFNNGYFYS YWNDGHGGVT YTNGPGGQFS
Tri ree RPAAEVESVA VEKRQTIQPG TGYNNGYFYS YWNDGHGGVT YTNGPGGQFS
Sch com .......... ....SGTPSS TGTDGGYYYS WWTDGAGDAT YQNNGGGSYT
Coc car .....ATDVS LVARQNTPNG EGTHNGCFWS WWSDGGARAT YTNGAGGSYS
Bac pum .........P AHARTITNNE MGNHSGYDYE LWKD.YGNTS MTLNNGGAFS
Clo ste ........PV LAGRIIYDNE TGTHGGYDYE LWKD.YGNTI MELNDGGTFS
Clo ace QKTYSAFNTQ AAPKTITSNE IGVNGGYDYE LWKD.YGNTS MTLKNGGAFS
Asp awa AASAGLLGHA FAAPVPQPVL VSRSAGINYV QNYNGNLADF TYDESAGTFS

        101                                               150
xyl20   VSWS.N.SGN FVIGKGWQYG AH.......N RVVNYN..AG AWQPNGNAYL
Bac sub VNWS.N.TGN FVVGKGWTTG SP.......F RTINYN..AG VWAPNGNGYL
Bac cir VNWS.N.TGN FVVGKGWTTG SP.......F RTINYN..AG VWAPNGNGYL
Str liv TQWT.N.CGN FVAGKGWSTG D......... GNVRYN...G YFNPVGNGYG
Str ec3 TQWT.N.CGN FVAGKGWGNG GR........ RTVRYS...G YFNPSGNGYG
Hum ins VRWR.N.TGN FVGGKGWNPG T........G RTINYG...G YFNPQGNGYL
Tri vir VNWS.N.SGN FVGGKGWQPG TK.......N KVINFS...G TYNPNGNSYL
Tri ree VNWS.N.SGN FVGGKGWQPG TK.......N KVINFS...G SYNPNGNSYL
Sch com LTWSGN.NGN LVGGKGWNPG AA.......S RSISYS...G TYQPNGNSYL
Coc car VSWG.S.GGN LVGGKGWNPG T........A RTITYS...G TYNYNGNSYL
Bac pum AGWN.N.IGN ALFRKGKKFD STRTHHQLGN ISINYN..AS .FNPSGNSYL
Clo ste CQWS.N.IGN ALFRKGRKFN SDKTYQELGD IVVEYG..CD .YNPNGNSYL
Clo ace CQWS.N.IGN ALFRKGKKFN DTQTYKQLGN ISVNYD..CN .YQPYGNSYL
Asp awa MYWEDGVSSD FVVGLGWTTG S........S NAISYS..AE YSASGSSSYL
```

fig.1 (cont)


```
          151        *                                        200
   xyl20  TLYGWTRNPL IEYYVVDSW. GSYRPT.... GDYRGSVYSD GAWYDLYHSW
 Bac sub  TLYGWTRSPL IEYYVVDSW. GTYRPT.... GTYKGTVKSD GGTYDIYTTT
 Bac cir  TLYGWTRSPL IEYYVVDSW. GTYRPT.... GTYKGTVKSD GGTYDIYTTT
 Str liv  CLYGWTSNPL VEYYIVDNW. GSYRPT.... GTYKGTVSSD GGTYDIYQTT
 Str ec3  CLYGWTSNPL VEYYIVDNW. GSYRPT.... GEYRGTVYSD GGTYDIYKTT
 Hum ins  AVYGWTRNPL VEYYVIESY. GTYNPGS..Q AQYKGTFYTD GDQYDIFVST
 Tri vir  SVYGWSRNPL IEYYIVENF. GTYNPST..G ATKLGEVTSD GSVYDIYRTQ
 Tri ree  SVYGWSRNPL IEYYIVENF. GTYNPST..G ATKLGEVTSD GSVYDIYRTQ
 Sch com  SVYGWTRSSL IEYYIVESY. GSYDPSS..A ASHKGSVTCN GATYDILSTW
 Coc car  AVYGWTRNPL VEYYVVENF. GTYDPSS..Q SQNKGTVTSD GSSYKIAQST
 Bac pum  CVYGWTQSPL AEYYIVDSW. GTYRPTG..A Y.KGSFYA.D GGTYDIYETT
 Clo ste  CVYGWTRNPL VEYYIVESW. GSWRPPG..A TPKGTITQWM AGTYEIYETT
 Clo ace  CVYGWTSSPL VEYYIVDSW. GSWRPPG..G TSKGTITV.D GGIYDIYETT
 Asp awa  AVYGWVNYPQ AEYYIVEDY. GDYNPCS..S ATSLGTVYSD GSTYQVCTDT
          201                                                 250
   xyl20  RYNAPSIDGT Q.TFQQYWSV RQQKRPTGS. .NVSITFENH VNAWGAAGMP
 Bac sub  RYNAPSIDGD RTTFTQYWSV RQSKRPTGS. .NATITFSNH VNAWKSHGMN
 Bac cir  RYNAPSIDGD RTTFTQYWSV RQSKRPTGS. .NATITFTNH VNAWKSHGMN
 Str liv  RYNAPSVEGT K.TFQQYWSV RQSKVTSGS. ..GTITTGNH FDAWARAGMN
 Str ec3  RYNAPSVEGT R.TFDQYWSV RQSKVI.GS. ..GTITTGNH FDAWARAGMN
 Hum ins  RYNQPSIDGT R.TFQQYWSI RKNKRV.... .GGSVNMQNH FNAWQQHGMP
 Tri vir  RVNQPSIIGT S.TFYQYWSV RRTHRS.... .SGSVNTANH FNAWAQQGLT
 Tri ree  RVNQPSIIGT A.TFYQYWSV RRNHRS.... .SGSVNTANH FNAWAQQGLT
 Sch com  RYNAPSIDGT Q.TFEQFWSV RNPKKAPGGS ISGTVDVQCH FDAWKGLGMN
 Coc car  RTNQPSIDGT R.TFQQYWSV RQNKRS.... .SGSVNMKTH FDAWASKGMN
 Cac pum  RVNQPSIIGI .ATFKQYWSV RQTKRT.... .SGTVSVSAH FRKWESLGMP
 Clo ste  RVNQPSIDGT .ATFQQYWSV RTSKRT.... .SGTISVTEH FKQWERMGMR
 Clo ace  RINQPSIQGN .TTFKQYWSV RRTKRT.... .SGTISVSKH FAAWESKGMP
 Asp awa  RTNEPSITGT S.TFTQYFSV RESTRT.... .SGTVTVANH FNFWAQHGFG

          251        *                             293
   xyl20  MGSSWSYQVL ATEGYYSSGY SNVTVW.... .......... ...
 Bac sub  LGSNWAYQVM ATEGYQSSGS SNVTVW.... .......... ...
 Bac cir  LGSNWAYQVM ATEGYQSSGS SNVTVW.... .......... ...
 Str liv  MGQFRYYMIM ATEGYQSSGS SNITVSG... .......... ...
 Str ec3  LGQFQYYMIM ATEGYQSSGS SNITVSG... .......... ...
 Hum ins  LG.QHYYQVV ATEGYQSSGE SDIYVQTH.. .......... ...
 Tri vir  LG.TMDYQIV AVEGYFSSGS ASITVS.... .......... ...
 Tri ree  LG.TMDYQIV AVEGYFSSGS ASITVS.... .......... ...
 Sch com  LGSEHNYQIV ATEGYQSSGT ATITVT.... .......... ...
 Coc car  LG.QHYYQIV ATEGYFSTGN AQITVNCP.. .......... ...
 Bac pum  MGKMYE.TAF TVEGYQSSGS ANVMTNQLFI GN........ ...
 Clo ste  MGKMYE.VAL TVEGYQSSGY ANVYKNEIRI GANPTPAPSQ SP*
 Clo ace  LGKMHE.TAF NIEGYQSSGK ADVNSMSINI GK........ ...
 Asp awa  .NSDFNYQVM AVEAWSGAGS ASVTISS... .......... ...
```

Bac sub = *Bacillus subtilis* (AC. P18429). Bac cir = *Bacillus circulans* (AC. P09850). Str liv = *Streptomyces lividans* (AC. P26220). Str ec3 = *Streptomyces sp.* EC3 (AC. S47512). Hum ins = *Humicola insolens* (AC. S43919). Tri vir = *Trichoderma viride* (AC. A44594). Tri ree = *Trichoderma reesei* (AC. P36217). Sch com = *Schizophyllum commune* (AC. P35809). Coc car = *Cochliobolus carbonum* (AC. Q06562). Bac pum = *Bacillus pumilus* (AC. P00694). Clo ste = *Clostridium stercorarum* (AC. JQ1935). Clo ace = *Clostridium acetobutylicum* (AC. P17137). Asp awa = *Aspergillus awamori* (AC. P33557).

SUBSTITUTE SHEET (RULE 26

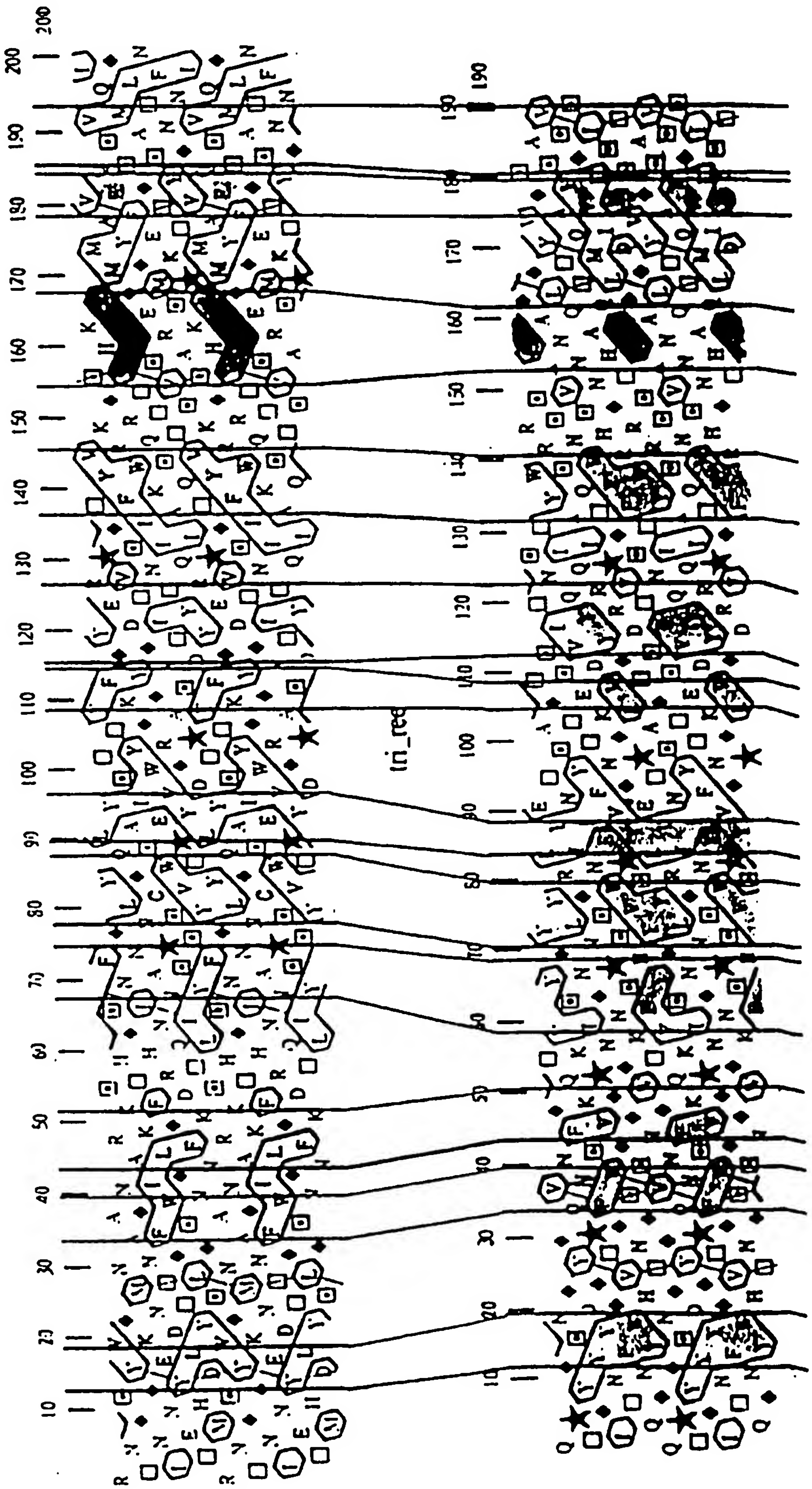

FIG. 2

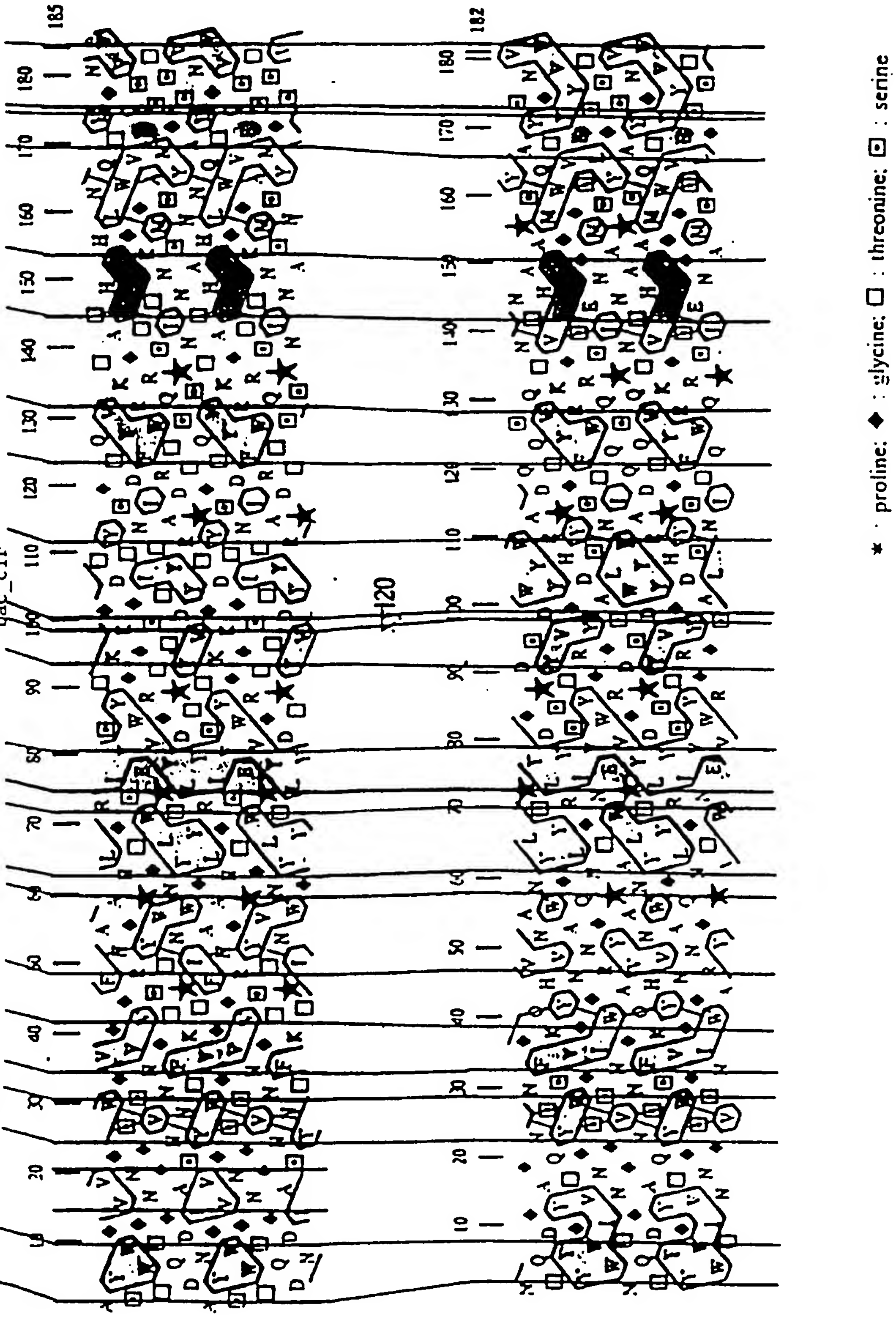

FIG.2 (cont)

```
                                                               αααααααα
1    NTYWQYWTDG IGYVNATNGQ GGNYSVSWSN SGNFVIGKGW QYGAHNRVVN 50
51   YNAGAWQPNG NAYLTLYGWT RNPLIEYYVV DSWGSYRPTG DYRGSVYSDG 100
101  AWYDLYHSWR YNAPSIDGTQ TFQQYWSVRQ QKRPTGSNVS ITFENHVNAW 149
     α
150  GAAGMPMGSS WSYQVLATEG YYSSGYSNVT VW 182
```

→ β-strands   ααα α-helix

FIG.3

INTERNATIONAL SEARCH REPORT

Inter al Application No
PCT/EP 96/04485

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/56 C12N15/70 C12N15/75 C12N9/24 C12N1/21
C12P21/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 13942 (INSTITUT NATIONAL DE LA RECHERCHE AGRONOMIQUE (INRA)) 20 August 1992	1-3
Y	see page 6, line 3 - page 7, line 16 see claims 3-11; examples 3,4 ---	4-7
Y	EP,A,0 634 490 (SOLVAY (SOCIÉTÉ ANONYME)) 18 January 1995 see page 4, line 6 - page 5, line 40 see page 13, line 54 - page 17, line 49 see examples 1-7,17,28,29,32,33,36,37 ---	4-7
Y	WO,A,95 18219 (GIST-BROCADES N.V.) 6 July 1995 see page 6, line 9 - line 28; examples 5-8 ---	4-7
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
7 January 1997	29.01.97

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Donath, C

Form PCT ISA 210 (second sheet) (July 1992)

Inter al Application No
PCT/EP 96/04485

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 507 723 (NOVO NORDISK A/S) 7 October 1992 see page 4, line 1 - page 7, line 19; examples 1,2 ---	4-7
Y	APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 60, no. 7, July 1994, pages 2252-2258, XP002022234 BABA, T. ET AL.: "Identification and characterization of clustered genes for thermostable Xylan-Degrading Enzymes, A-Xylosidase and Xylanase, of Bacillus stearothermophilus 21" see the whole document ---	4-7
A	APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 61, no. 6, June 1995, pages 2420-2424, XP002022235 TABERNERO, C. ET AL.: "Cloning and DNA sequencing of xyaA, a gene encoding an endo-A-1,4-Xylanase from an alkalophilic Bacillus strain (N137)" see the whole document ---	1-7
A	ENZYME MICROB.TECHNOL., vol. 8, May 1986, pages 309-314, XP002022236 GRÜNINGER, H. AND FIECHTER, A.: "A novel, highly thermostable D-xylanase" see the whole document -----	1-3

1

INTERNATIONAL SEARCH REPORT

Inte.   ..nal Application No
PCT/EP 96/04485

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9213942	20-08-92	FR-A- 2672300	07-08-92
		AU-B- 666301	08-02-96
		AU-A- 1376192	07-09-92
		BG-A- 97987	25-04-94
		BR-A- 9205551	07-06-94
		CA-A- 2101063	02-08-92
		CZ-A- 9301500	19-01-94
		EP-A- 0573536	15-12-93
		HU-A- 64392	28-12-93
		JP-T- 6506107	14-07-94
		SK-A- 82093	07-09-94
EP-A-0634490	18-01-95	GB-A- 2279955	18-01-95
		AU-A- 6743294	27-01-95
		BR-A- 9402834	13-06-95
		CA-A- 2128050	16-01-95
		FI-A- 943389	16-01-95
		JP-A- 7067637	14-03-95
		NO-A- 942652	16-01-95
		NZ-A- 260989	28-08-95
WO-A-9518219	06-07-95	AU-A- 1415095	17-07-95
		BR-A- 9405934	26-12-95
		EP-A- 0686193	13-12-95
		FI-A- 953920	21-08-95
		JP-T- 8507221	06-08-96
		NO-A- 953312	19-10-95
EP-A-0507723	07-10-92	CA-A- 2106484	03-10-92
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